PLANT RESPONSE TO SALINE SUBSTRATES

V. CHLORIDE REGULATION IN THE INDIVIDUAL ORGANS OF HORDEUM VULGARE DURING TREATMENT WITH SODIUM CHLORIDE

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Summary

This is a study on the regulation of chloride concentrations in *H. vulgare* at the early tillering stage, when grown on media of high sodium chloride concentration. ^{36}Cl was used during certain periods to determine retranslocation.

Chloride, which had been absorbed either during the first 5 days, or between the fifth and tenth day of sodium chloride treatment, was not lost from the whole plant during subsequent periods, and this chloride moved to the shoot. Over the same period there was further chloride uptake from the medium.

Developed leaves lost only small amounts of chloride by retranslocation and the concurrent intake far exceeded this loss. Evidence is presented that low concentrations in younger leaves are due to low mobility of chloride already taken up by older organs of the shoot, and not to a chloride exclusion by younger organs.

It is concluded that chloride contents within the shoot of *H. vulgare* are regulated at two locations:

(1) During transfer from the root to the shoot.

(2) During translocation from older to younger organs.

In growing organs, ion concentrations depend not only on the rates of ion uptake but also on relative growth rates, i.e. ion concentrations at any one time are higher at low than at high relative growth rates.

This interaction between growth and ion concentration is discussed in relation to ion imbalance, osmotic adjustments, and salt tolerance.

I. INTRODUCTION

On media of high ionic concentration plant tolerance to internal ion unbalance is usually limited; therefore even the most tolerant species must regulate their ionic composition.

This paper discusses regulation of chloride concentrations in *Hordeum vulgare* which was used in earlier studies on salt tolerance (Greenway 1962*a*, 1962*b*; Greenway and Rogers 1963). *H. vulgare* does not increase in succulence on media of high sodium chloride concentration (Eaton 1942; Greenway 1962*a*) and has no salt glands. Thus, ion regulation in the shoot can be achieved only by ion export to the medium via the roots (Hylmö 1953), or by limitation of ion transfer to the shoot.

Most species grown on saline media only regulate their ion balance to a certain extent (Bernstein and Hayward 1958). Additional regulation might be achieved in certain organs, cells, or parts of cells, but this would increase the ion unbalance

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of other parts. For shoots of *H. vulgare* such a localization in ion concentration has been found (Greenway 1962*b*; Greenway *et al.* 1965). Young leaves, inflorescences, and developing grain had ion balances similar to those of plants grown on media low in sodium chloride, but older organs contained very high chloride and sodium, and very low potassium concentrations.

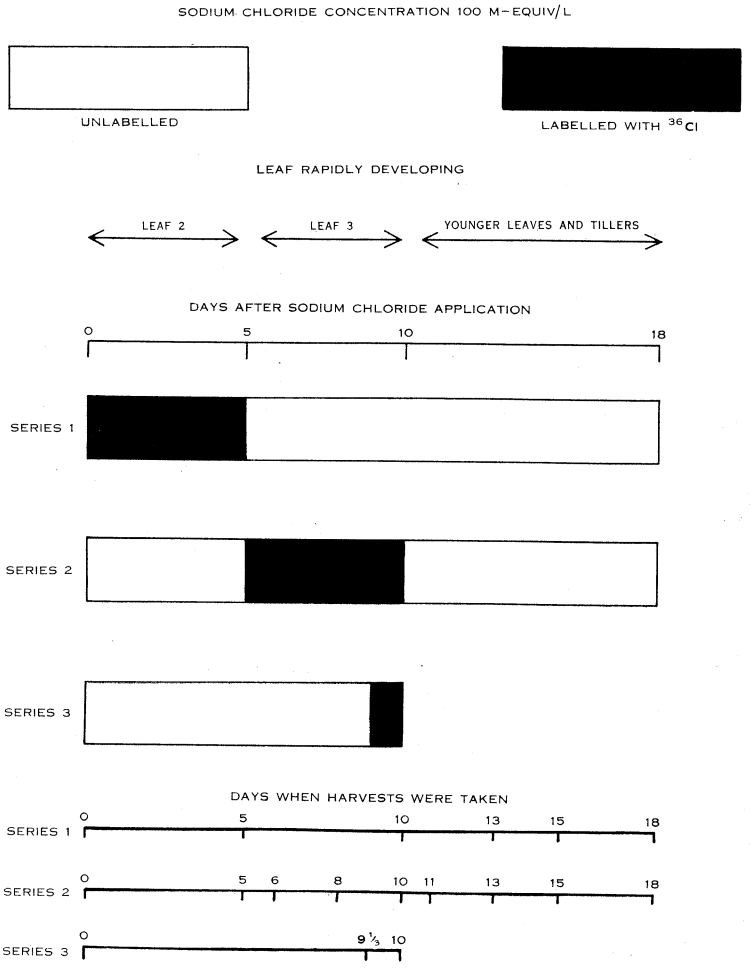


Fig. 1.—Periods when media were labelled with ^{36}Cl, and days of harvest for each of the three series. ^{36}Cl concentrations were 10 μc/l in series 1 and 3, and 5 μc/l in series 2.

II. Methods

(*a*) *General*

Chloride export and redistribution within plants was determined during different periods of plant development by addition of ^{36}Cl to the medium and subsequent replacement by non-labelled solutions of the same sodium chloride concentration.

H. vulgare cv. Bolivia was grown as described previously (Greenway 1962*b*) except that no chloride was supplied prior to sodium chloride treatment. To adjust the plants to saline media, sodium chloride treatment was at 50 m-equiv/l for the first day, and subsequently between 98 and 102 m-equiv/l throughout the rest of the experiment.

Sodium chloride treatment was commenced after the first leaf had completed its phase of rapid development. The experiment was divided into three series in which ^{36}Cl was added (as $Na^{36}Cl$) at different stages of plant development (see Fig. 1), i.e. the rapidly developing leaf was the second oldest leaf in series 1 and the third oldest in series 2. In series 3, leaf 4 had just commenced development.

During harvests (at times shown in Fig. 1), the plants were separated into roots, stem, leaf 1 (the oldest leaf), leaf 2, leaf 3, leaf 4, leaf 5, tiller 1 (the oldest tiller), tiller 2, and tiller 3. Sheaths were included with their individual leaves.

Chloride taken up by the plant as a whole or its individual parts during the different periods of the experiment is denoted as follows:

Cl_{0-5} for chloride absorbed during the first 5 days of sodium chloride treatment (series 1).

Cl_{6-10} for chloride absorbed between the sixth and tenth day (series 2).

Cl_{10} for chloride absorbed during the tenth day (series 3).

Rates of chloride retranslocation were calculated by three different methods (cf. Greenway and Pitman 1965):

(1) Uncorrected estimates, from the changes with time in total and labelled chloride within developed organs.

(2) Improved estimates, similar to (1) but corrected for variability in Cl_{0-5} or Cl_{6-10} at the day of ^{36}Cl removal.

(3) Assessed estimates; the above estimates underestimate the rate of retranslocation because developed leaves, while exporting, will also receive from other plant parts some chloride which had been absorbed by the plant as a whole during preceding periods. A maximum estimate of this chloride intake by developed leaves can be made, which then leads to another estimate of rate of retranslocation (for details see Greenway and Pitman 1965).

(*b*) *Harvesting and Analytical Techniques*

There were eight replicates in the glasshouse, four of these being pooled at harvest, so that all measurements were on two replicates each consisting of four separately grown plants.

Plants were transferred from labelled to non-labelled solutions and after 3 min all roots and stem bases were rinsed with a jet of non-labelled solution before being transferred to new non-labelled solutions. These were again replaced at $1\frac{1}{2}$ hr, 1 day, and 2 days after removal from ^{36}Cl-containing medium, then at 4-day intervals.

During harvest the roots were rinsed for 3 min in cold distilled water. All samples were dried rapidly at 75°C.

Samples were ground in a Wiley micromill so as to pass through a 40-mesh sieve and the ground samples were spread uniformly on flat aluminium planchets. Radioactivity was counted with a mica end-window Geiger-Müller tube. The counting rates were corrected for self-absorption, which had been established from a standard series of the same plant material with known ^{36}Cl concentration (method by Woolley, personal communication).

Total chloride was determined by electrometric titration (Best 1950).

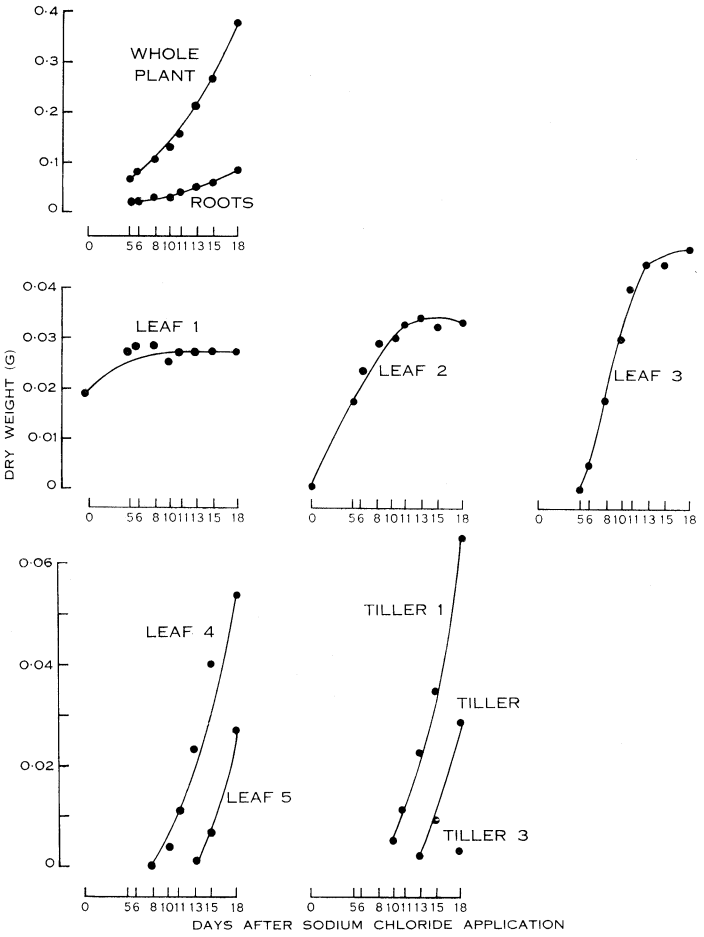


Fig. 2.—Dry weight changes for the whole plant and its individual organs.

III. Results

(a) *Chloride Uptake and Retranslocation in the Plant as a Whole and in Its Individual Organs*

(i) *Dry Weight.*—Growth is highly relevant to the interpretation of ion uptake and retranslocation. Dry weight changes are shown in Figure 2, and the pattern of

development was typical for the early tillering stage of *H. vulgare*, i.e. there were leaves at all stages of development, from fully mature to recently initiated.

The oldest leaf (leaf 1) had completed its development on the fifth day of treatment (Fig. 2). Leaf 2 developed rapidly between days 0–5 and more slowly between days 6–10, thereafter it represented a fully developed leaf. Leaf 3 developed rapidly between days 5 and 13, and then much more slowly. Organs younger than leaf 3 (leaves 4 and 5, tillers 1 and 2) all commenced their rapid development during the later part of the experiment (after tenth day).

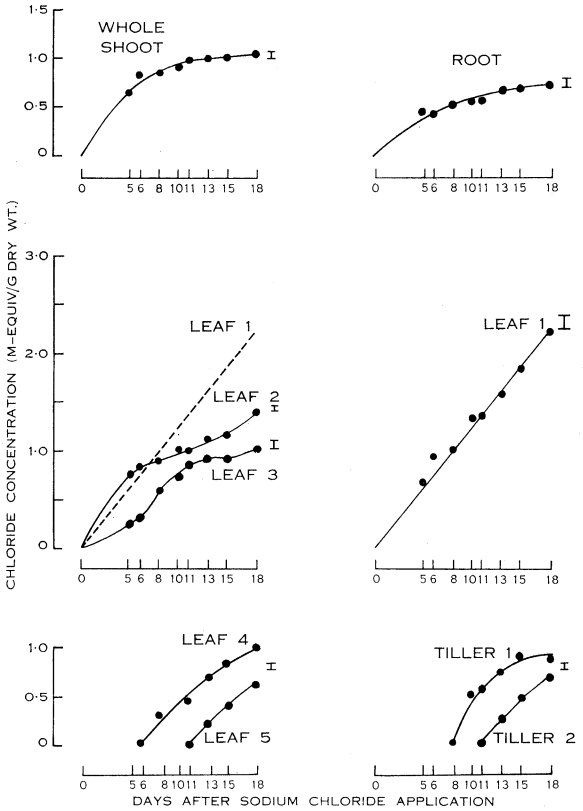


Fig. 3.—Chloride concentration of the whole plant and its individual organs. Least significant differences shown are at $P = 0{\cdot}05$.

(ii) *Chloride Concentration.*—Chloride concentrations of the whole plant and its individual organs are shown in Figure 3. The distribution of chloride within the shoot was typical (Greenway 1962*a*, 1962*b*, 1963; Greenway *et al.* 1965), with much higher chloride concentrations in older than in younger organs. Rates of increase in chloride concentration were also similar to previous experiments (Greenway 1962*b*). In the shoot rate of increase was rapid for the first 5 days with no further increase in concentration after the thirteenth day of treatment with sodium chloride. The concentration of chloride in the roots showed a similar trend except for the slow rise till the end of the experiment.

The oldest leaf (leaf 1) showed a linear increase in chloride concentration with time. The rate decreased in leaf 2 when this leaf had attained about half its final dry weight (day 5). Subsequently chloride concentration increased only slowly till day 15 (the fifth day after completion of leaf development), but rose again towards the end of the experiment (days 15–18).* Rapidly developing organs (leaves 3, 4, and 5; tillers 1 and 2) showed the rather rapid rise in chloride concentration typical for the early stage of leaf development. However, leaf 3 and tiller 1 entered a slow phase of increase in chloride concentration towards the end of the experiment, i.e. the rate after day 13 is similar to that of leaf 2 between days 5 and 15 (Fig. 3).

TABLE 1

RATE OF CHLORIDE UPTAKE* BY PLANT PARTS OF DIFFERENT AGE

Days after Sodium Chloride Application	Chloride Uptake (m-equiv/g/day)		
	Leaf 3	Leaf 4 (youngest leaf)	Tiller 1
5–6	0·33	—	—
6–8	0·40	0·32†	—
8–10	0·24	0·30	0·50†
10–11	0·33	0·42	0·35
11–13	0·09	0·32	0·34

* Uptake of leaf 1 (oldest leaf) between 8 and 13 days was 0·13 m-equiv/g/day.

† These organs commenced development between 6–8 days (leaf 4) and 8–10 days (tiller 1), so that the actual period of chloride reception was shorter than the 2 days used in the calculations. The rates are, therefore, underestimates and must have been considerably higher.

In the present experiment plants were harvested at close intervals and this enabled a detailed determination of the rise in chloride concentration during rapid leaf development. Both leaf 4 and tiller 1 (the oldest tiller) showed a rapid rise in chloride concentration during the very early period of leaf development (6–8 and 8–10 days respectively), and a lower rate of increase during the major period of rapid leaf growth.

A lower rate of increase in chloride concentration with time could be due to declining rates of ion uptake. Rates of chloride uptake for leaves 3 and 4 and tiller 1 are given in Table 1. There is no change with time for leaf 4 and a decrease for tiller 1. It is concluded that there must have been decreases in the actual rates of chloride uptake, because the rate of chloride uptake during the initial period of leaf development would have been underestimated (see second footnote of Table 1).

(iii) *Chloride Uptake and Redistribution for Shoots and Roots.*—Figure 4 shows, for shoots and roots, the total chloride content, and the redistribution of chloride

* That this is a real rise in chloride concentration, upon aging of the leaf, is supported by the similar trends in chloride and sodium concentrations during leaf development observed in other experiments (Greenway 1962*b*, 1963; Greenway *et al.* 1965).

supplied between days 0–5 (Cl_{0-5}) and days 6–10 (Cl_{6-10}), respectively. Pronounced increases in chloride content of the whole plant, shoots, and roots occurred throughout the experiment. The plant retained all the chloride absorbed prior to day 5 (Fig. 4). Roots lost about 70% of their Cl_{0-5} during the subsequent 5-day period, most of which was transferred to the shoot and not to the medium (Fig. 4). Similar trends were found for Cl_{6-10} (Fig. 4, 10–18 days).

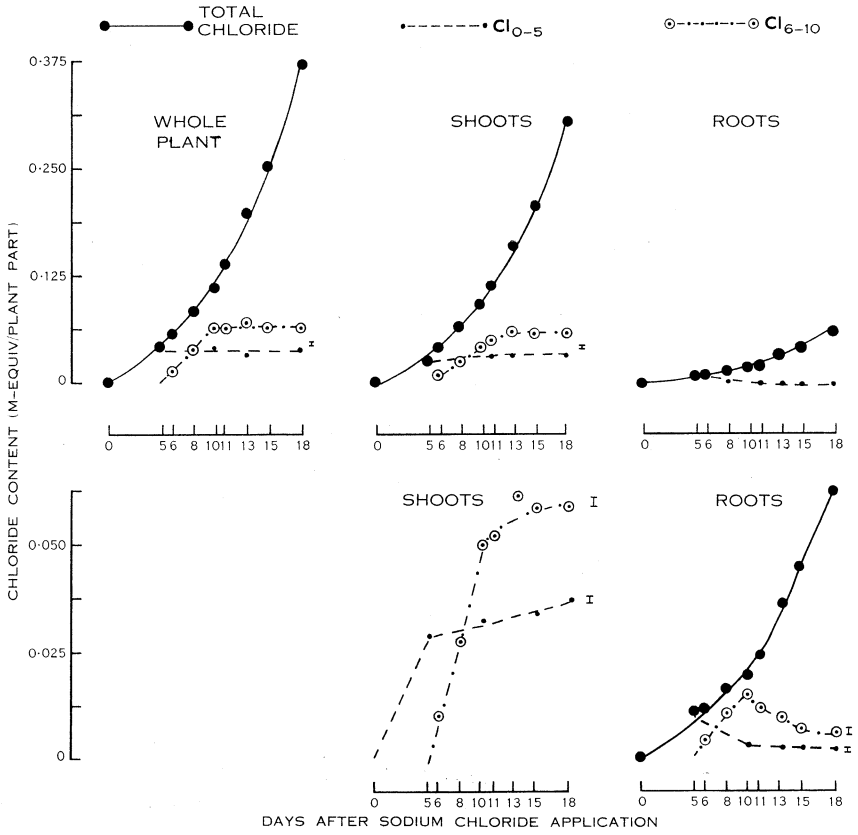


Fig. 4.—Total chloride content and redistribution of chloride supplied between days 0–5 (Cl_{0-5}) and between days 6–10 (Cl_{6-10}) for the whole plant, shoots, and roots.

(iv) *Chloride Uptake and Retranslocation for the Individual Organs of the Shoot.*—The methods measured net rather than total retranslocation of ions absorbed during a particular period (Greenway and Pitman 1965). Cl_{0-5}, Cl_{6-10}, and total chloride for individual organs of the shoot are shown in Figure 5 and rates of retranslocation for Cl_{0-5} are given in Table 2. Usually uncorrected and improved estimates were very similar, and the assessed estimates were of value immediately after removal of tracer from the medium.

In all individual organs of the shoot, rapid increases in total chloride occurred [Fig. 5(*a*)]. Chloride supplied prior to day 5 was retranslocated from the two oldest leaves (leaves 1 and 2)—from leaf 1 slowly over the whole period (5–18 days) and from leaf 2 only after day 13 [Fig. 5(*a*); Table 2]. Thus retranslocation of chloride

absorbed between days 0–5 began only some time after the leaves had completed their development. Chloride supplied between days 6–10 was not only retranslocated

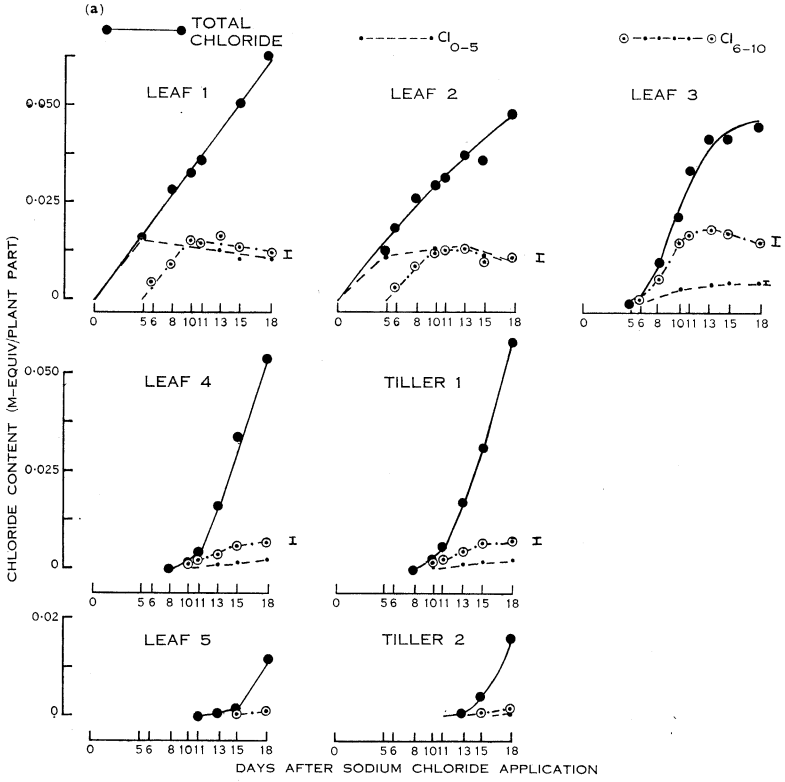


Fig. 5.—(*a*) Total chloride content and net retranslocation of chloride supplied between days 0–5 (Cl_{0-5}) and days 6–10 (Cl_{6-10}) for individual parts of the shoot. Least significant differences shown are at $P = 0{\cdot}05$. (*b*) Cl_{0-5} in individual plant parts as a percentage of total Cl_{0-5} in the whole plant, and showing its redistribution after day 5 (series 1).

from the oldest leaves (leaves 1 and 2), but also from the more recently developed leaf 3 [Fig. 5(*a*), 13–18 days].

Chloride lost from the roots and developed leaves moved into newly developing organs such as leaf 3 between the fifth and tenth day of treatment, and leaves 4 and 5 and tillers 1 and 2 after the eighth day [Fig. 5(*b*)].

Chloride retranslocation, although small relative to the amounts present in the older organs, contributed appreciably to the intake by newly developing organs. For example, Cl_{0-5} contributed 12% to the total chloride in the young organs at day 10 [leaf 4 and tiller 1, Fig. 5(*a*)]. Chloride in the developed organs (leaves 1, 2,

TABLE 2

RATES OF RETRANSLOCATION OF CHLORIDE ABSORBED DURING THE FIRST 5 DAYS

Plant Part	Days after Sodium Chloride Application	Rate of Chloride Retranslocation (μ-equiv/plant part/day)		Total Plant Chloride‡ (μ-equiv.)
		Uncorrected Estimate*	Improved Estimate†	
Leaf 1	5–8	0·125	1·1§	1·6§
	8–10	0·37	0·75	1·7
	10–13	0·32	0·82	2·5
	13–18	0·39	0·32	1·3
Leaf 2	5–8	Gain	Gain	—
	5–10	Gain	Gain	—
	10–13	Gain	Gain	—
	13–18	0·45	0·45	1·5

* Calculated from the primary data on chloride content.

† Adjusted by assessments of inter-plant variability (Greenway and Pitman 1965).

‡ Assuming that chloride was retranslocated at the same specific activity as the leaf as a whole.

§ Assessed estimate—see Section II.

and 3) consisted of about 45% Cl_{0-5}, so the amount of chloride received via retranslocation by the young organs would be $(100/45 \times 12) = 26\%$.* This is a minimum estimate because chloride recently taken up by developed leaves might be translocated at a faster rate than the major pool of chloride in these leaves [see Section IV(*b*)].

(*b*) *Chloride Uptake over Short Periods* (*Series 3*)

In series 3, chloride uptake from the medium was measured over 7- and 26-hr periods on the tenth day of sodium chloride treatment. Chloride concentrations at this time are shown in Figure 3 and uptake from the medium in Figure 6. In the whole shoot, chloride uptake from the medium (Cl_{10}) was linear with time. However, it increased with time in young organs (leaf 4 and tiller 1), while it decreased in developed organs (leaf 1 and, particularly, leaf 2).

* An estimate of 30% was obtained for day 15, when release from roots was negligible.

IV. Discussion

Regulation of ion concentrations is important to plant growth on saline media, because of osmotic pressure adjustments and possible ion toxicities.

(a) *Chloride Uptake and Retranslocation in the Whole Plant, Shoots, and Roots*

During the early tillering stage, *H. vulgare* did not lose any appreciable amounts of chloride, which had been absorbed during preceding periods (Fig. 4). Thus no chloride loss occurred despite subsequent conditions which would have favoured exchange, i.e. the further chloride uptake by the plant led to very high chloride

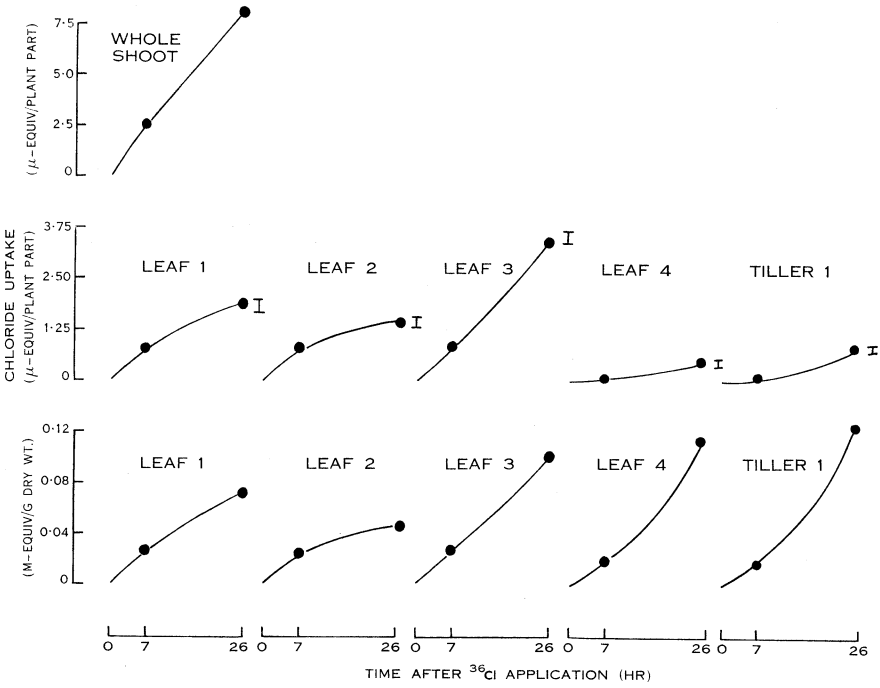


Fig. 6.—Chloride uptake from medium during the tenth day of sodium chloride treatment (series 3). Least significant differences shown ($P = 0{\cdot}05$) are at 26 hr (leaves 1, 2, and 3) and at 7 hr (leaf 4, tiller 1).

concentrations in a number of organs (Figs. 3 and 4). There was therefore no evidence for regulation by ion circulation through the plant and a return to the medium of the excess, i.e. those ions not absorbed by individual cells of the shoot, as suggested by Hylmö (1953). Chloride of the root does not exchange markedly with the medium.* Chloride absorbed by the root during a particular period was lost only slowly during the subsequent period (Fig. 4, 5–10 and 10–15 days) and mostly transferred to the shoots, not to the medium (Fig. 4). Though some of the root chloride might exchange rather freely with the medium the major part was apparently not involved. The shoots showed a distinct further intake of chloride which had been supplied to the

* The rinsing period of 3 min during harvest would have removed most of the readily exchangeable chloride.

plant as a whole during a preceding period (Fig. 4). Thus for *H. vulgare* the only possible mechanism for chloride regulation in the shoot appears to be a pronounced limitation of chloride intake via the roots. Under conditions of high transpiration, the transpiration stream contained only 3 m-equiv/l chloride at a medium concentration of 100 m-equiv/l (Greenway 1965).

(b) Chloride Uptake and Retranslocation in Individual Organs of the Shoot

Chloride retranslocation has been established for plants of low chloride content, e.g. in soy beans after foliar application (Gage and Aranoff 1960), and in tomatoes after chloride was exhausted from the medium (Woolley, Broyer, and Johnston 1958). These experiments indicated a high rate of chloride retranslocation. In the present experiments, with plants of high chloride content, quantitative estimates of retranslocation were compared with rates of concurrent chloride intake. The developed leaves showed a much slower retranslocation than intake, i.e. between days 13–18 the oldest leaf (leaf 1) lost 1·3 μ-equiv/day,* but it took up 5·3 μ-equiv/day.

Throughout leaf development chloride concentration will be determined by intake, retranslocation, and volume changes; how these regulate the chloride concentration in a leaf is summarized schematically in Figure 7 and Table 3.

TABLE 3

SUGGESTED MECHANISM FOR CHLORIDE REGULATION OF INDIVIDUAL LEAVES

Phase	Chloride Derived from:	Chloride Uptake	Regulation of Chloride Concentration	Manner in which Chloride Concentration is Regulated
1—Rapidly growing leaf	(i) Medium (ii) Older organs of the shoot	High	Good	Rapid growth, i.e. dry weight increments
2—Developed leaf	Medium	Low*	Good	Slow net uptake } Retranslocation smaller than uptake
3—Maturing leaf	Medium	Intermediate*	Poor	Rapid net uptake } Retranslocation smaller than uptake

* Differences between phases 2 and 3 might be due to different rates of retranslocation; these different rates are not indicated in Figure 7, which only shows retranslocation rates based on measurements of Cl_{0-5} and Cl_{6-1}.

During rapid growth (phase 1) rate of chloride uptake was high and there was no measurable export. The rise in chloride concentration was rapid at first but then slowed down, mainly due to the diluting effects of rapid increments in dry weight. After phase 1 the rise in chloride concentration will solely depend on net intake, i.e. the difference between total intake and retranslocation [Fig. 7(*b*)].

This net intake was rather small once the leaf had developed, resulting in a slow rise of chloride concentration, but was high in the maturing leaf (phase 3) leading to a

* Based on improved estimates.

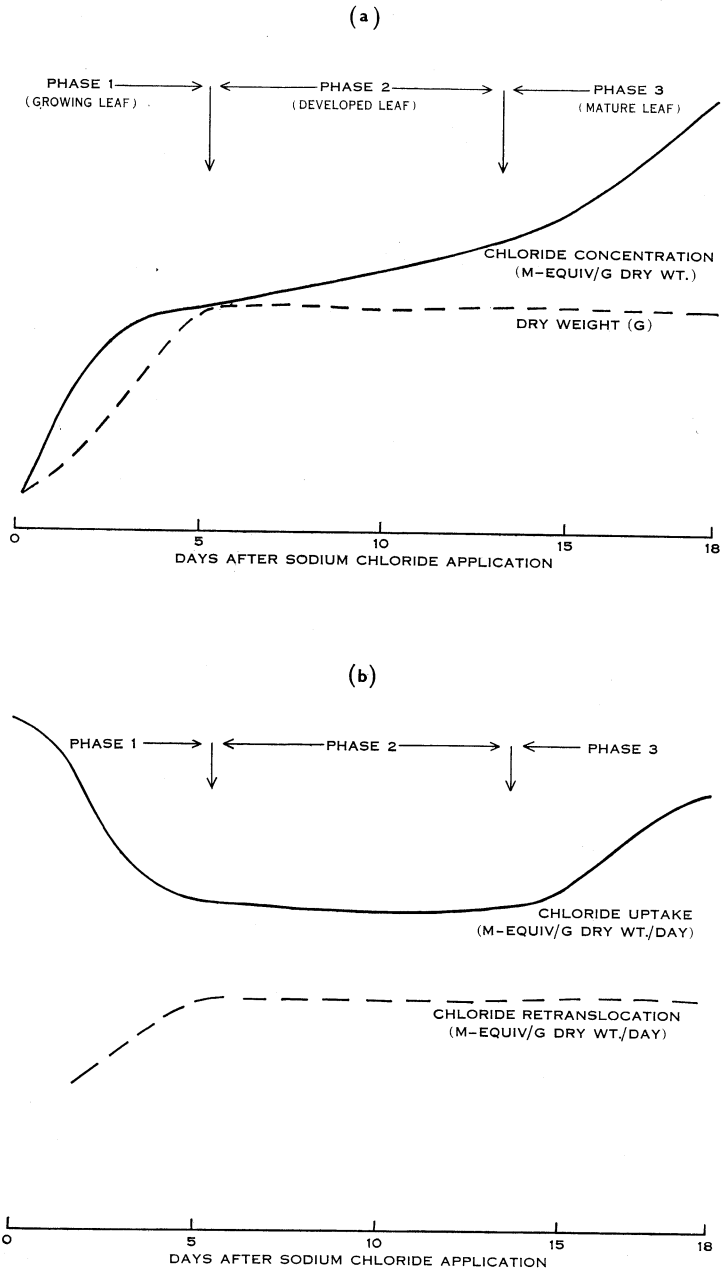


Fig. 7.—(*a*) Dry weight changes and changes in chloride concentration throughout leaf development. Data from Figures 2 and 3 and from Greenway (1962*b*) and Greenway *et al.* (1965). (*b*) Chloride uptake (see Table 1 and Figure 6) and retranslocation (see Table 2 and Figure 5) throughout leaf development. The diagrams are based on media containing sodium chloride at concentrations ranging from 50 to 150 m-equiv/l.

rapid rise in chloride concentration. Though leaves of different age absorbed chloride at different rates over a 24-hr and longer periods (Figs. 6 and 7), their rates of Cl_{10} intake were very similar during the first 8 hr of measurement (tenth day of sodium chloride treatment, Fig. 6), i.e. Cl_{10} intake decreased with time in developed organs (leaves 1 and 2) but increased in developing organs (leaf 4 and tiller 1). Such a pattern would be consistent with a rapid retranslocation of chloride recently taken up by developed organs (i.e. between 0–8 hr, Fig. 6), probably by interchange between xylem and phloem (Bollard 1960). Because of the high chloride concentrations already in the leaf cells of the developed organs, their ion uptake is likely to be slow (Briggs, Hope, and Robertson 1961) and retranslocation of recently arrived chloride might, therefore, be rapid.

(*c*) *Mechanisms of Chloride and Sodium Regulation within the Shoot*

For potassium, changes in the rate of retranslocation during leaf development are best interpreted by assuming differences in competition between the translocation system and the cells of the individual leaves (Greenway and Pitman 1965).

Ion retranslocation might be determined by:

(1) Exclusion of chloride by cells of young organs;

(2) Immobility, due either to retention by older organs or to a limited capacity of the transport system (for further details see Greenway *et al.* 1965).

For plants of high chloride and sodium content, the very high concentrations in older leaves could be due to two alternative mechanisms (Greenway 1962*b*). Firstly, chloride and sodium might be highly mobile within the shoot. The young organs would then require an ability to exclude chloride, such an ability being lost when the leaf matures, leading to very high chloride and sodium concentrations in the older leaves. In this case condition (1) would be the rate-limiting step in net chloride retranslocation. Secondly, there might be a restricted mobility of chloride within the shoot. Then the continued chloride uptake, via the transpiration stream, would build up very high chloride concentrations in the older leaves, i.e. the organs which had received chloride for the longest period and in which growth did not limit the rise in chloride concentration.

The degree of immobility in the shoot can be assessed from the ratio of the amount of ion absorbed after day 5 to the amount of ion absorbed between days 0–5, and these ratios for chloride and sodium are shown in Table 4. If there were a rapid circulation the various organs would tend to equilibrate and therefore show very similar ratios. However, at all harvests the ratios were much higher in young than in old organs, so circulation was not very rapid.

A low chloride export from young leaves was also indicated by chloride uptake shortly after its application to the medium (during tenth day of sodium chloride treatment, Fig. 6). The pattern of uptake in leaves of different age was consistent with a rapid retranslocation from older to younger organs [see Section IV(*b*)], and not with export from younger organs.

TABLE 4

MOBILITY OF CHLORIDE AND SODIUM IONS BETWEEN VARIOUS PARTS OF H. VULGARE PLANTS

Mobility indicated by ratio of ions absorbed after day 5 to those absorbed between days 0–5

Distribution of Chloride Ions (Series 1)						Distribution of Sodium Ions*				
Plant Part	Time in Days after ^{36}Cl Removal				Least Significant Difference ($P = 0{\cdot}05$)	Plant Part	Time in Days after ^{22}Na Removal			Least Significant Difference ($P = 0{\cdot}05$)
	5	8	10	13			2	5	10	
Leaf 1	0·9	2·5	3·8	4·8	1·6	Leaf 1	0·03	0·3	0·6	0·8
Leaf 2	1·4	1·7	2·0	2·9		Leaf 2	0·2	0·6	2·3	
Leaf 3	5·6	9·2	8·9	9·3		Leaf 3	1·3	3·8	7·4	
Leaf 4	11·2	12·5	17·7	22·2		Leaf 4	4·9	9·1	15·0	7·2
Leaf 5	—	8·1	18·3	22·5		Leaf 5	—	1·3†	20·0	
Tiller 1	7·7	10·9	17·1	21·1	3·9	Leaf 6	—	—	1·0†	—
Tiller 2	—	5·1	21·1	19·5		Tiller 1	2·8	6·8	14·4	7·2
Tiller 3	—	—	—	19·6		Tiller 2	—	1·7†	15·1	—
Stem	7·4	11·9	10·6	26·0		Tiller 3	—	—	3·2†	—
Roots	6·1	13·7	22·7	34·9		Sheath 1	0·1	0·6	2·5	0·8
						Sheath 2	1·0	2·5	5·7	
						Younger sheaths	1·5	13·0	15·0	—
						Roots	0·9	1·9	4·6	—

* Experiment 3 of Greenway *et al.* 1965. Sodium chloride (concentration 100 m-equiv/l) labelled with ^{22}Na for first 5 days of the experiment.

† These values (based on single measurements) are doubtful because counting rates were very low (between 1–3 counts/min with background at 20 counts/min). If confirmed, the low ratios would show that very young organs receive a large proportion of their sodium via retranslocation from other organs.

Net retranslocation rates would depend on the concentrations in the young organs, if uptake by the sink determined the rate of retranslocation. The effect of the concentration of chloride and sodium in the young leaves can be assessed by considering previous experiments in which sodium chloride was removed from the medium (Greenway 1962*b*). For the younger organs removal of sodium chloride resulted in much lower chloride and sodium concentrations than did continuous sodium chloride treatment, yet in both treatments there were similar rates of chloride as well as sodium retranslocation (Table 2; cf. Greenway 1962*b*, Greenway *et al.* 1965).

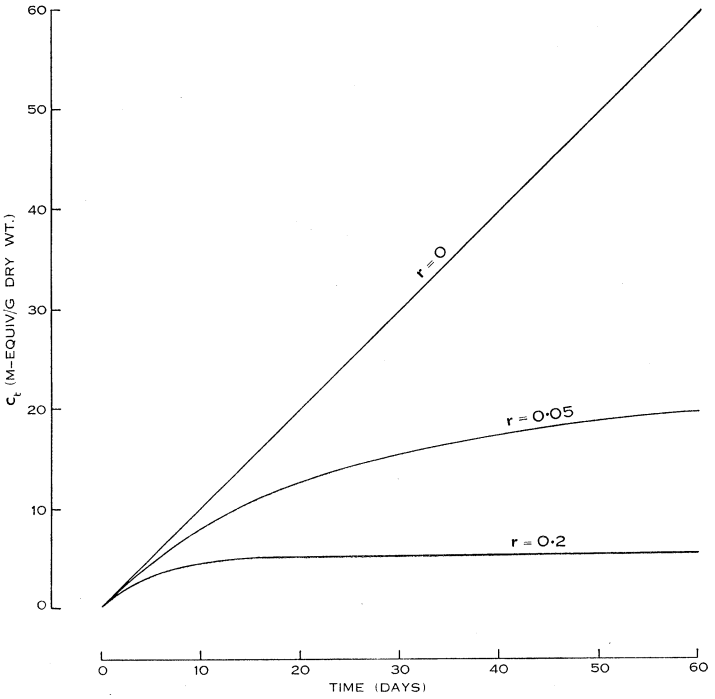


Fig. 8.—Change of ion concentration with time after sodium chloride application (C_t, calculated according to equation (1), see p. 520) at three relative growth rates. Note that units of the ordinate have to be multiplied by i, the rate of ion uptake in m-equiv/g/day which, for example, would be different in various species.

The observations in the three preceding paragraphs suggest that chloride and sodium are not very mobile within shoots of *H. vulgare* treated with sodium chloride, and that the sink did not determine retranslocation. If so, the slow retranslocation would be due to either a strong retention by the cells of the older organs or to a limited transport via the retranslocation system.

It is suggested that for *H. vulgare*, grown on media high in sodium chloride, the low chloride and sodium concentrations of young shoot organs are achieved by selectivity at two locations:

(1) In the roots, through which only small amounts are transferred to the shoots.

(2) In older leaves, from which only relatively small amounts are retranslocated.

V. General Discussion on Ion Regulation by Growth

(a) *Regulatory Effect by Growth*

It has been shown that the total chloride content of the shoot was regulated only during the intake via the roots (Fig. 4). Yet the chloride concentration of the shoot did not change after 13 days of sodium chloride treatment (Fig. 3) and for both chloride and sodium a steady concentration was maintained until the onset of senescence (Greenway *et al.* 1965). It can be shown that this attainment of a constant ion concentration is due to diluting effects of growth, i.e. to increases in volume. Thus for the following conditions: ion concentration $C = 0$ at $t = 0$, where t = time in days; rate of ion uptake (in m-equiv/g/day) $= i$ $(=1/W.\mathrm{d}I/\mathrm{d}t$, where I = ion content and W = dry weight) = constant; and relative growth rate (in g/g/day) = r = constant; then the dry weight as a function of time will be

$$W_t = W_0 \mathrm{e}^{rt},$$

and the ion uptake by the whole plant at time t will be

$$i^* = iW_t.$$

Hence the ion concentration at time t is

$$C_t = \left[\int_0^t iW_0\,\mathrm{e}^{rt}\,\mathrm{d}t\right] \Big/ W_0\,\mathrm{e}^{rt}$$

$$= (i/r).(1-\mathrm{e}^{-rt}). \qquad (1)$$

Therefore,

$$\mathrm{d}C/\mathrm{d}t = i\,\mathrm{e}^{-rt}. \qquad (2)$$

Figure 8 shows the ion concentrations over a 60-day period for two relative growth rates commonly found in plants, and for a relative growth rate of zero. At this latter growth rate the ion concentration increases linearly with time ($\mathrm{d}C/\mathrm{d}t = i$), but at any other relative growth rate the concentration rises rapidly at first and eventually reaches a constant value.

For the present experiment chloride concentration, relative growth rate, and chloride uptake (m-equiv/g/day) are presented in Table 5. There was very little change in relative growth rate an ion uptake during the first 15 days and the observed 1·0 m-equiv/g at day 15 agrees with the calculated value of 0·94 m-equiv/g. Similar agreement between observed and calculated concentrations was found for chloride and sodium in *H. vulgare* between early tillering and development of inflorescence (Greenway *et al.* 1965).

Within the shoot, the regulatory effect of growth occurred in developing organs, where concentrations increased in a pattern consistent with equation (1) (Table 6). In the developed organs dilution would have occurred if retranslocation had been rapid, as for example in plants of low phosphate status (Williams 1948).

(*b*) *Relevance to Salt Tolerance*

Regulatory effects of growth are relevant to the effects of sodium chloride on potassium concentrations and should always be taken into account. For example, in *Atriplex hastata*, Black (1956) found that potassium concentrations decreased after prolonged sodium chloride treatment, but only below sodium chloride concentrations of 200 m-equiv/l. Black assumed two types of potassium uptake, with only that

TABLE 5

CHLORIDE INTAKE, CHLORIDE CONCENTRATION, AND RELATIVE GROWTH RATE FOR THE WHOLE SHOOT

Series 1 and 2

Time after Sodium Chloride Application (days)	Chloride Intake (m-equiv/g dry wt./day)	Chloride Concentration (m-equiv/g dry wt.)	Relative Growth Rate (g/g/day)
0–5	0·16	0·66	0·16
5–10	0·16	0·92	0·16
10–15	0·18	1·00	0·16
15–18	0·13	1·06	0·13

at lower sodium chloride concentrations being reduced by sodium. However, growth was reduced at sodium chloride concentrations higher than 200 m-equiv/l, and this could have stabilized potassium concentrations, even if uptake (m-equiv/g/unit of time) had been further reduced.

TABLE 6

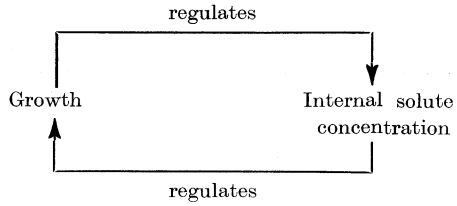
RELATIVE GROWTH RATES AND CHLORIDE CONCENTRATION IN INDIVIDUAL LEAVES AT DAY 15

Leaf No.	Relative Growth Rate (g/g/day)	Chloride Concentration (m-equiv/g dry wt.)
1	0	1·83
2	0	1·15
3	0	0·93
4	0·28	0·84
5	0·85	0·42

Adjustment of plants to saline media would also be influenced by interrelationships between growth and ion concentration. Growth rates will often be decreased, accentuating the increase in ion concentrations within the plant. If ion unbalance effects are important this process might continue, i.e. increase in internal ion con-

centration giving decreased growth rate, giving increase in ion concentration until concentrations reach lethal levels.

In many species, however, ion concentrations are maintained at such a level that internal diffusion pressure deficits increase by the same amount as the osmotic pressures of the medium, suggesting that growth reductions on saline media are not due to inadequate osmotic pressure adjustments (Eaton 1942; Bernstein 1961, 1963; Slatyer 1961; and Eaton and Bernandin 1964). However, it is suggested here that the rate of the solute accumulation process might be insufficient to give adequate osmotic adjustments in growing cells. The manner in which growth and ion concentration might interact, resulting in a restoration of the osmotic pressure differential between cell and medium, is indicated in the scheme below. (It is assumed that the rate of ion uptake is limited and does not give sufficiently rapid osmotic pressure adjustment.)



The sequence of events is as follows:

(1) external osmotic stress
↓
(2) reduced growth
↓
(3) rapid increase in internal solute concentration [equations (1) and (2) and Fig. 8]
↓
(4) at least partial recovery of growth
↓
(5) slowing down of increase in internal solute concentration [equations (1) and (2)].

This sequence results in attainment of a steady state where this interaction between growth and ion concentration leads to "adequate" internal osmotic pressure adjustments, i.e. the same osmotic pressure differentials between cell and medium as at low salinity; yet this would only be achieved at the expense of rapid growth.

As shown in this scheme the system would reach a steady state where the interaction between growth and ion concentration is optimal for the medium concerned. As a result "adequate osmotic pressure adjustments"* could be attained in shoot organs and whole shoots, despite rates of solute accumulation which are inadequate to sustain rapid growth. The suggestions receive support from the high rate of electrolyte absorption in most halophytes as reported by Adriani (1958), and merit further investigation.

*"Adequate", now in the sense that the osmotic pressure would have increased as much as that of the medium, not as in Bernstein's usage of "adequate" for rapid growth.

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